

1 **Revisiting mechanisms and functions of prenatal hormone-mediated maternal effects**
2 **using avian species as a model**

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ABSTRACT

Maternal effects can adaptively modulate offspring developmental trajectories in variable but predictable environments. Hormone synthesis is sensitive to environmental factors, and maternal hormones are thus a powerful mechanism to transfer environmental cues to the next generation. Birds have become a key model for the study of hormone-mediated maternal effects because the embryo develops outside the mother's body, facilitating the measurement and manipulation of prenatal hormone exposure. At the same time, birds are excellent models for the integration of both proximate and ultimate approaches, which is key to a better understanding of the evolution of hormone-mediated maternal effects. Over the past two decades, a surge of studies on hormone-mediated maternal effects has revealed an increasing number of discrepancies. In this review, we discuss the role of the environment, genetic factors and social interactions in causing these discrepancies and provide a framework to resolve them. We also explore the largely neglected role of the embryo in modulating the maternal signal, as well as costs and benefits of hormone transfer and expression for the different family members. We conclude by highlighting fruitful avenues for future research that have opened up thanks to new theoretical insights and technical advances in the field.

Keywords: parental effects, phenotypic plasticity, context dependency, parent-offspring conflict, steroids, yolk hormones

Hormone-mediated maternal effects: bridging traditional discipline boundaries

The optimal phenotype depends on the environment an individual is going to encounter. Because phenotypic plasticity is not infinite and often associated with significant costs [1], and developing young have limited information about the environment, parental guidance of offspring developmental trajectories is assumed to be favoured by natural selection in variable but predictable environments [2, 3]. Over the past 20 years parental effects have witnessed a steep increase in attention, both in the context of understanding individual differences in human health and disease, the capacity of organisms to respond to global change, the limited explanatory power of genetic information alone and the surge of interest in epigenetics. In this review we focus on prenatal parental effects mediated by hormones of maternal origin. Hormones not only provide a well-described and measureable pathway for parental effects, especially in the sensitive early developmental period, but are also an ideal tool to translate environmental cues from the mother to the offspring as environmental conditions strongly affect maternal hormone production and transfer.

Although studied in various taxa, from insects to mammals, oviparous species have been a key model for research into hormone-mediated maternal effects because, unlike in viviparous species, the embryo develops outside of the mother's body. This facilitates the measurement and manipulation of embryonic hormone exposure without interfering with the mother, whereas such confounding interference is inevitable in viviparous taxa. Moreover, after egg laying, there is much less hormonal communication between mother and offspring possible than in placental animals, facilitating interpretation. However, as we will see below, recent findings suggest that both mothers and offspring may still influence the fate of maternal hormones transferred to the yolk, in which the extra-embryonic membranes may function as a 'quasi'-placenta.

For eco-evolutionary studies, birds are especially well suited because their survival and reproductive success can be relatively easily studied in the field, in contrast to many other taxa. Moreover, systematic differences in avian yolk hormone concentrations within clutches (i.e. eggs of the same nest), between clutches, between mothers, and between

species have been demonstrated, and the effect of a variety of ecological variables on hormone transfer (i.e. in the quantity of maternally produced hormone that ends up in eggs, which is influenced by maternal hormone production and active or passive transfer processes) documented [4]. As a consequence the study of hormones in bird eggs has strongly contributed to ultimate explanations for the transfer of maternal hormones to the next generation, with relevance for other fields, including human health and disease [5, 6].

After the initial discovery of gonadal hormones in bird eggs [7], the field has seen a surge of publications. However, as more studies are published, the patterns also become more complex with an increase in discrepancies among studies and species. Rather than seeing these discrepancies as something negative, they are intriguing and should stimulate new ideas and research approaches, as scientific progress often comes from challenging established hypotheses rather than supporting them. Therefore, goals of this paper are to highlight potential explanations for observed discrepancies among studies, discuss recent discoveries and technical advances, and outline promising avenues for future research.

Understanding discrepancies: the role of pleiotropy and context dependency

Hormones are well known for having pleiotropic effects, influencing a range of different aspects of an individual's phenotype, and synchronizing changes in brain and body periphery [8]. The first studies on maternally derived hormones in bird eggs showed that maternal yolk androgens boost early offspring growth, either directly or indirectly via stimulating begging behaviour [7, 9]. Although this pattern has been confirmed in several studies and species, the opposite pattern or no effect on growth have been reported in the course of time (reviewed in [4]). In addition, effects of maternal yolk androgens on a wide range of other physiological, behavioural, morphological and life history traits have been reported, including, for example, immune suppression, an increase in metabolic rate, modulation of antioxidant status, hormone receptor densities, the expression of secondary sexual characters, exploratory behaviour or natal dispersal (reviewed in [4, 10, 11]). Several of these effects,

such as immune suppression, modulation of oxidative status or an increase in metabolic rate, are likely to be costly for the offspring, potentially resulting in a trade-off between beneficial and detrimental effects of hormone transfer.

This has two consequences: First, facing this trade-off, mothers may transfer hormones to their offspring differently depending on contextual cues that influence these costs and benefits. For example, in an environment where parasite abundance is high or food availability is low, the immunological or metabolic costs of exposure to elevated androgen concentrations during development may outweigh the benefits in terms of enhanced growth, and mothers may therefore reduce the amount of hormones they transfer [12]. Similarly, females may transfer higher concentrations of androgens to their last laid egg when food conditions are favourable to increase this chick's success in the competition with earlier hatched and stronger siblings, whereas they may do the opposite when food is scarce to facilitate brood reduction. In line with this hypothesis, an effect of food availability on the transfer of hormones to eggs has been observed in some bird species (e.g. [13, 14]). Other studies, however, found no effect of food availability on egg hormone concentrations (e.g. [15, 16]). These discrepancies might be due to differences in the stability and / or predictability of food abundance in different study systems [17]. Indeed, if there is low temporal autocorrelation between food availability during egg laying and chick rearing, mothers cannot predict the conditions their offspring are going to encounter, and anticipatory maternal effects would not be expected to evolve [2].

Second, pleiotropic effects of yolk androgens may influence the outcomes of egg hormone injection experiments depending on the prevailing context under which they are performed. Support for this hypothesis was found in a recent experiment in rock pigeons (*Columba livia*) which lay a modal clutch size of two eggs, with the second hatching later than the first and containing substantially higher androgen concentrations [18]. Manipulation of both yolk androgen content and food availability in a full-factorial design showed that elevated yolk androgen levels had clear beneficial effects under favourable food conditions, facilitating survival of both the first and second chick, but strong negative effects under poor

food conditions, facilitating brood reduction in times of food shortage. The effects of yolk hormone manipulations may also depend on the egg's position in the laying order (and thus the chick's position in the sibling rivalry), where the positive effects are present only when the chick is in a competitive disadvantage [19, 20].

Overall, a better understanding of the mechanisms underlying the pleiotropic effects hormones have on various body traits and functions will be key to advance our understanding of hormone-mediated trait integration, as well as trade-offs and constraints associated with maternal hormone transfer. For example, it is still unclear if pleiotropy is the consequence of independent effects of hormones on multiple targets, or if hormones affect a single regulatory system with downstream effects on various traits. Under the former scenario, constraints on evolution and limitations for the mother to influence offspring phenotype are likely less pronounced than under the latter. However at the same time, the latter may reduce the risk of improper integration of individual traits into a single phenotype (see [21] for further discussion). Gene expression analysis (i.e. whole transcriptome sequencing) in combination with gene set enrichment and pathway analysis is a promising approach to gain insights into the mechanisms underlying pleiotropic effects of maternal hormones, and we encourage studies on the mechanisms and functions of hormone-mediated maternal effects to incorporate such technologies.

In addition to environmental factors discussed above, genetic factors (such as offspring sex) can influence the expression of hormone mediated-maternal effects and mediate their fitness consequences. Interestingly, there is no general pattern which sex is more strongly affected by maternal yolk hormones across bird species (reviewed in [22, 23]). This variation is at least partly explained by differences in the strength of selection acting on male and female body size, respectively, with growth of female offspring being more strongly boosted by maternal yolk hormones in species where selection favours a large female body size [22]. It suggests that (natural and / or sexual) selection may shape the sex-specific sensitivities to maternal yolk hormones within species, and highlights that an ultimate perspective is key to understanding the seemingly inconsistent patterns across species. It

also highlights that wrong conclusions may be drawn when not accounting for offspring sex. Yet, to date, only a minority of studies on hormone-mediated maternal effects have explicitly modelled such sex-specific consequences [23]. Molecular sexing methods are now available for most bird species [24] and we urge researchers to consider the sex of their focal individuals when studying phenotypic consequences of maternal hormone transfer.

As exposure to elevated maternal hormone concentrations is associated with costs for the offspring (see above), only offspring of highest genetic quality may be able to bear these costs. This might explain why females deposit higher androgen concentrations in eggs sired by an attractive male [25], as well as discrepancies in the effects of egg hormone manipulations across broods. Egg hormone manipulations in combination with artificial insemination with sperm from high and low quality males would allow to shed light on this hypothesis.

Complex eggs: the challenge of performing biologically meaningful manipulations

Interactions among hormonal and non-hormonal egg constituents

Most experimental studies on hormone-mediated maternal effects have manipulated one or two related hormones in isolation [23], which allows pinpointing the effect of a particular hormone on offspring phenotype, but may lead to unnatural imbalances within eggs, and thus create artefacts. This is the case if different yolk constituents are mutually co-adjusted within eggs, as is observed in a number of bird species [26-30]. Selection may favour co-deposition of different egg components if they have opposite, compensatory effects on offspring phenotype (reviewed in [31]). For example, hormone-induced changes in growth and metabolism may increase levels of free radicals that can cause physiological damage if not buffered adequately by antioxidants [32-35]. Similarly, exposure to high androgen concentrations during development may cause immunosuppression [36], which might render offspring particularly susceptible to infection unless compensated for by alternative protection

mechanisms such as the transfer of maternal immunoglobulins [27]. Thus, by only increasing hormone levels, without simultaneously increasing other co-adjusted egg constituents, an unnatural imbalance may be created that may negatively affect offspring development. To our knowledge, only one study has experimentally tested this hypothesis by simultaneously manipulating two co-adjusted yolk constituents (i.e. yolk testosterone and carotenoid concentrations) in a full-factorial design [37]. This study found evidence that imbalances in the egg have negative consequences for the chicks [37], highlighting that single-egg-constituent manipulations, as is usually done, might disturb a fragile equilibrium within eggs. A multivariate approach to quantifying and manipulating egg constituents is therefore recommended, which will be facilitated by on-going technical advances (e.g. in mass spectrometry or proteomics), but will nevertheless remain challenging. As an example of such a multivariate approach, a recent study used high-performance liquid chromatography with tandem mass spectroscopy (LC-MS-MS) to simultaneously quantify 27 yolk steroids from the eggs of seven shrubland bird species [38]. Such approaches open up new avenues for the quantification of hormonal profiles and their association with ecological variables both within and across taxa.

Artefacts may not only arise when egg constituents are manipulated in isolation but also when hormones doses for injection experiments are not carefully chosen (i.e. are not biologically and ecologically meaningful). Dose-response effects have been documented for several hormones [23, 39], yet specific dose-response curves are missing for most study systems. An additional complication is that it is not trivial to quantify concentrations of hormones in eggs (see section '*What is in an egg? Challenges and advances in the measurement of yolk hormones*' below), which makes it difficult to determine biologically and ecologically meaningful doses for injection experiments. Together, these technical difficulties and gaps in our knowledge significantly hamper further progress in our understanding of functional, ecological and evolutionary consequences of prenatal hormone exposure.

In addition, a significant hurdle in manipulative studies is that we can only increase embryonic exposure to hormones, but not decrease it. Currently the dynamics of blockers of

relevant receptors or hormone conversions in the egg are unknown, and they may not only affect maternal hormone conversion or action, but also the embryo's endogenous production, which can start early during incubation (see below).

Finally, testing whether hormone-mediated maternal effects are anticipatory (i.e. prepare the offspring for the ecological conditions they are going to encounter) requires a full-factorial match-mismatch design, in which the manipulated environmental variable is ecologically relevant and shows a pattern of temporal autocorrelation that allows parents to 'predict the future' [40]. Unless these conditions are met, no benefits of matched conditions are expected to be observed.

Which hormone matters?

Traditionally, there has been a strong focus on maternally transferred androgens [7]. Yet, mothers transfer a range of other hormones that may mediate (anticipatory) maternal effects. Gonadal hormones, such as testosterone, androstenedione or progesterone are produced in the follicle wall very close to the ovum [41]. They are at least partly independently regulated from hormone concentrations in the mother's circulation (reviewed [41]), suggesting that females can flexibly bestow their eggs with different concentrations of gonadal hormones depending on the ecological context. Indeed, both within [10] and across species [38, 42] variation in yolk androgen deposition can be partly explained by ecological variables.

Although concentrations of progesterone in yolks is often much higher than that of testosterone or androstenedione [43, 44], this hormone has received comparably little attention to date. Progesterone has been shown to vary across the laying sequence and in relation to a range of ecological variables [45-47] and it has been suggested to affect the chicks' fear behaviour [48] and facilitate brood reduction [46]. However, no study has yet experimentally and selectively manipulated this hormone in the egg and more studies are required to fully understand interactions with other egg components and the role of this hormone in mediating maternal effects [28].

Another important class of maternal hormones to which developing young may be exposed to prenatally are glucocorticoids, such as corticosterone and cortisol, which are produced in the mother's adrenals, and have thus to be transported via the mother's circulation to the follicles, and then enter the yolk. Whether an increase in circulating glucocorticoids in the mother automatically leads to increased concentrations in the yolk is as yet clear, and rather than having direct effects on offspring development, circulating glucocorticoids may affect the embryo via indirect pathways, such as suppressed maternal nutrient provisioning (reviewed in [49]). In mammals there is evidence that the placenta might act to prevent maternal glucocorticoids to reach the embryo [50]. To what extent this is the case in oviparous species is as yet unclear (but see below).

A third class of hormones that may mediate (anticipatory) maternal effects are thyroid hormones. Although produced by the mother's thyroid gland distant from the ovaries, there is some evidence that mothers may be able to regulate concentrations of these hormones in their eggs independently from their own circulation [51]. These hormones are particularly interesting because their production requires iodine that cannot be synthesized *de novo* and which availability in the environment may be limited. If so, this would induce an additional trade-off for the mother between allocating limited thyroid hormone to herself or her offspring. Thyroid hormones are important for normal development of the brain, peripheral tissues, thermoregulation and metabolism [52]. After the initial studies of McNabb and her coworkers more than twenty years ago [53, 54], these maternally transmitted hormones have, until very recently, received little attention. The few experimental studies performed, however, show clear effects of prenatal thyroid exposure on hatching success, early growth, and thyroid hormone production [55, 56].

In addition to the hormones discussed above there is a wide range of other hormones in the yolk (e.g. growth hormones and other peptide hormones) that have received little to no attention to date. A better understanding of the role of these understudied hormones in mediating maternal effects may thus be promising avenue for future research and proteomics

and metabolomics approaches might be particularly fruitful for explorative studies into such understudied egg components.

What is in an egg? Challenges and advances in the measurement of yolk hormones

In the past, most studies have relied on analytical methods such as radioimmunoassay (RIA) or enzyme immunoassay (EIA) to determine hormone concentrations in eggs. It now becomes clear that these methods yield often too high hormone concentrations [57, 58]. This is probably not surprising, as most assays have been designed for particular substrates (mostly plasma) and species, and not for lipophilic egg yolk. Furthermore, it has recently been shown that a wide array of steroids found in egg yolk can cross-react with antibodies used in these assays, leading to artefacts when the extraction, clean-up and separation processes preceding RIA / EIA are inadequate [59-61]. These preparatory processes are often not properly conducted, and rarely validated for the target hormones under study. This raises serious questions about which hormones, and in which concentrations, are actually present in eggs. It also raises concerns about the dosages used in egg injection experiments, as they are often calibrated on the basis of RIA / EIA results. This is particularly concerning because the effects of most hormones are strongly dose-dependent [23, 39]. Thereby, such technical issues can contribute to discrepancies in findings across studies. The re-evaluation of previously reported hormone-deposition patterns in relation to environmental variables and the quantification of hormone concentrations using modern, sensitive liquid chromatography–mass spectrometry approaches (i.e. LC-MS-MS or GC/LC-M(M)S) is therefore recommended (as used e.g. in [38, 62-64]).

Finally, a recent study revealed that maternal hormones are already partly metabolized in the oviduct after ovulation [59]. This provides another hurdle for the interpretation and experimentation of maternal hormones, as levels measured at oviposition may not adequately reflect what the mother initially deposited in the egg.

Hormone-mediated maternal effects – a family affair

Parental effects are assumed to have evolved as a mechanism that allows parents to adaptively shape offspring phenotype in variable, but predictable environments [3]. As a consequence most behavioural ecology research on hormone-mediated maternal effects is based on the assumption that changes in maternal hormone transfer in response to environmental factors lead to higher offspring fitness [10]. However, this assumption ignores the possibility of conflicts of interest within families [65, 66]. A whole-family perspective is therefore essential to further our understanding of the selective forces that shape variation in maternal hormone transfer as well as the response to these maternal cues [67].

Conflicts between mothers and their offspring

If maternal and offspring interests are aligned, offspring should be sensitive and reactive to maternal cues. However, maternal and offspring interests are not always aligned, resulting in mother-offspring conflict [65-67]. For example when maternal investments favour future reproduction over current reproduction, current offspring will pay a fitness cost. Similarly, when mothers favour certain offspring within a reproductive event (e.g. offspring early in the laying sequence [68]), the disfavoured offspring will pay a fitness cost. If such conflicts arise, parental effects will not increase the fitness of (all) offspring, as is indeed found in many studies [17, 49, 69], and natural selection will thus favour resistance mechanisms that help offspring to ignore or modify maternal manipulation attempts [67, 70].

Hormone receptors for androgens and glucocorticoids are present before the embryo starts to produce its own hormones, both in mammals [71] and birds [72, 73], suggesting adaptation to receive the maternal hormonal signals. Interestingly, part of these receptors (as well as hormone-metabolising enzymes) are present in the placenta in mammals and in the extra-embryonic membranes of egg laying species [74, 75], indicating homologous functions of both tissues. Hormone receptors are also found in the brain of developing embryos. However, to what extent maternal hormones reach the brain is, surprisingly, as yet not clear [76, 77].

Although the machinery is thus in place to perceive maternal hormonal cues, there is also growing evidence that embryos rapidly and heavily metabolize maternal hormones in the egg [62, 78, 79]. Intriguingly, most of these metabolites are (supposedly) inactive forms of the free steroids, such as conjugated forms or etiocholanolone in the case of androgens [80]. It has been suggested that this conversion may facilitate uptake by the embryo, after which the inactive hormones might be converted back to the original, biological active form [78, 81]. Alternatively, these metabolites may have an as yet unknown biological effect on the embryo, such as erythropoiesis [80]. Clearly, these are intriguing avenues for future studies. The active role of the embryo opens up the possibility that they are not just passive responders to the maternal signal but may influence how they react to it and thus how maternal effects are expressed. For example, other egg components may inform the embryo about ecological relevant cues such as food availability, parasite abundance, or the position in the laying sequence and therefore the level of sibling competition. Based on such information the embryo may differentially metabolise maternal hormones. A first, recent study testing this hypothesis found that rock pigeon embryos in the second egg, (being the last laid egg with relatively high testosterone concentrations, see earlier) metabolise yolk androgens significantly more than embryos in the first egg [62]. Understanding if this is just a consequence of the second egg having much higher natural maternal testosterone concentrations, or if this increased metabolism is rather related to the egg's position in the laying order will be an important next step and will help to clarify the adaptive significance of embryo-induced hormone metabolism.

Although these empirical studies thus show that there is scope for offspring to influence the expression of hormone-mediated maternal effects, theoretical models predict that if conflict occurs, mothers will likely have the upper hand or that the transgenerational information transfer will partly or fully break down [66]. More likely, both parties thus benefit on average from hormone-mediated signalling systems, ensuring their stability [66]. Together, these recent findings demonstrate how ultimate thinking and theory can prompt and inform studies on mechanisms, and vice versa.

Costs for the mother

In addition to potential conflicts of interest between mothers and their offspring, quantifying the costs of hormone transfer for the mother is key to a better understanding of the evolution of hormone-mediated maternal effects. So far, very few studies have quantified the fitness consequences of maternal hormone transfer for the mother, and, to our knowledge, none has quantified the costs of maternal *plasticity* in hormone transfer in response to environmental variation.

Artificial selection is an elegant but underused experimental tool to reveal such maternal costs, and costs associated with different reproductive strategies in general [82, 83].

Okuliarová et al. [84] used this approach to create genetic lines of Japanese quail (*Coturnix japonica*) that differ in the amount of testosterone females transfer to their eggs. This selection experiment revealed that an increase in yolk testosterone concentrations can be achieved without a correlated increase in testosterone concentrations in the female's circulation, highlighting that the mother's hormone levels do not constrain the evolution of yolk testosterone transfer. Rather, correlative work in the same species suggests that yolk testosterone transfer is associated with metabolic costs for the female [85], which might ultimately underlie the negative association between yolk testosterone transfer and female longevity observed in wild birds [86]. Thus, yolk testosterone transfer is associated with significant metabolic and longevity costs for the female, which ensure that egg hormones are an honest signal. These costs increase the stability of hormone-mediated transgenerational signalling systems [66, 67, 69] and create a trade-off between current and future reproductive investment that is central to models of life history evolution [87].

Mechanistic studies are now required to gain insights into the proximate basis of variation in maternal hormone deposition and its associated costs, and artificial selection experiments [84, 88] in combination with gene expression profiling [89] would be a particularly fruitful approach to shed light on these questions.

The role of fathers

Conflicts cannot only arise between mothers and their offspring but also between mothers and fathers [90]. In species with biparental care, mothers and fathers disagree over the amount of care each parent provides to the offspring: The fitness benefits of parental investment depend on the summed contribution of both partners. However, the costs of parental investment are paid by each parent individually [65]. Thus, conflicts over parental care arise because each individual benefits if the partner works disproportionately hard [65]. It has been hypothesised that females may manipulate males into increasing their contribution to parental care by transferring high concentrations of yolk androgens into their eggs, and thereby enhance offspring begging behaviour ('Manipulating Androgen Hypothesis (MAH)'; [67, 91]). Several studies have tested this hypothesis, but found no evidence for this [92-96]. One study even found that mothers are more sensitive to yolk hormone-mediated begging signals than fathers [97], which is the opposite of what is predicted under MAH [91]. There was some support for MAH in a study on yellow-legged gulls (*Larus michaellis*), which found that yolk testosterone affects a specific component of the chicks' begging calls to which fathers are particularly sensitive to [98]. Although this study did not analyse provisioning rates of the two parents, it suggests that at least in some species females can shift the outcome of the sexual conflict over care in their favour by means of maternal hormones. Methodological improvements of measuring parental provisioning may however be required to detect such manipulative effects [99].

Whereas hormone-mediated maternal effects may affect the father's behaviour (see above), inversely males also have the potential to influence their partner's transfer of hormones to the eggs. Several studies have reported that females deposit higher concentrations of androgens into their eggs when mated to an attractive, supposedly high quality male, and this effect appears to be particularly strong in species where only females provide parental care (reviewed in [25]). This finding is in line with the idea that females should invest more in broods with a higher reproductive value [87, 100], assuming that 'more is better', which is not necessarily the case for yolk androgens [10]; see also above). To our

knowledge, the adaptive value of this differential allocation of yolk androgens in response to partner attractiveness has not been experimentally tested to date.

Besides such indirect effects of male attractiveness on egg hormone transfer, males may directly influence their offspring's response to maternally-derived hormones. In mammals, it is well documented that paternally imprinted genes expressed in the offspring can influence the mother's prenatal resource provisioning [101]. In birds, the potential for such male manipulation of female care has been questioned because the embryo develops outside of the mother's body, and females transfer all the resources they provide prenatally to the developing young before (paternally-imprinted) genes in offspring are expressed [102, 103].

Besides genomic imprinting, males may be able to modify the offspring's response to maternal cues through, for example, the transfer of variable epigenetic marks [104] or the transfer of non-coding RNA or proteins via sperm [105]. Exploring such paternal effects, their interaction with maternal effects, and their consequences for the resolution of within-family conflicts will be one of the most exciting avenues for future research in our opinion.

In conclusion, although the study of hormone-mediated maternal effects has exposed an increasing number of discrepancies over the last two decades, recent technical and theoretical advances have revealed that these are not simply uninteresting inconsistencies among studies, but rather intriguing phenomena that deserve further research. There is a diversity of environmental, social and genetic contexts that modify the transfer of maternal hormones, their expression as well as their fitness consequences, as summarized in Fig. 1. To complicate things further, these interactions can come to expression in a range of different ways, from nullifying to strengthening and even reversing phenotypic consequences, and from acting as permissive or compensatory effects [40]. Identifying such contexts that explain discrepancies among studies will thus be an important next step. In addition, recent technical advances have revealed that the embryo itself may play an active role in modifying the maternal signal. This may have consequences for the resolution of parent-offspring conflicts, and is an intriguing avenue for further research, as is the potential

417 role of the father and interaction among different egg components in shaping the expression
418 of hormone-mediated maternal effects.

419 Author contribution:

420 TG and BT wrote the paper. Parts of the context dependency, hormone conversion and
421 receptor activity sections are based on the PhD thesis of BYH and NK, respectively, under
422 supervision of TG.

423

424 The authors have no conflicting interests.

425

426 Acknowledgement

427 The authors thank two anonymous referees and the editor for constructive comments on the
428 manuscript.

429 Figure legend

430 Figure 1: Interactions between mother, father, embryo and the environment in shaping the
431 expression of hormone-mediated maternal effects.

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